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FINAL REPORT BIODETECTION GRINDER

By

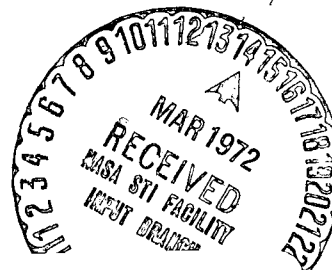
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ABSTRACT

This report summarizes the work on the Biodetection Grinder. It includes development of the prototype grinder, second generation grinder and the production version of the grinder. Tests showed the particle size distribution was satisfactory and biological evaluation confirmed the tests.

Recognition is given to Mr. W. A. Pesch for the development of the concept, grinder parts and other valuable contributions to the program.

Reference is made to the other reports used in preparing the final report, namely: Hayes Technical Reports MD-265, 268, and 507.

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INTRODUCTION

In order to determine the presence of microbial life in outer space and to determine whether spacecraft components sent into outer space are sterile, a method of checking for embedded as well as surface microorganisms is necessary. Embedded microorganisms survive in some hard materials to great depths and sampling has to be made at the proper depth or cross section for accurate evaluations. Also, some aerospace materials have to be reduced to 1 - 8 micron size in order to obtain the best biological assay.

Conventional grinding cannot be used to obtain these sizings because the high energy level results in heating the particles high enough to kill living organisms. Also, the shearing action contributes to the reduction of microbial life. It is not feasible or may not be possible to accurately measure the maximum temperature of the micro particles because of size and cooling rate. Therefore, empirical methods are used to establish the best sampling techniques.

One of the present methods of sampling aerospace materials for detection of embedded organisms utilizes the crushing action of a Pica Mill Blender. Destruction of microorganisms using this method is high and decreases the accuracy of the tests.

With this background data, a program was initiated for the development of a sampling device to reduce the particles to the required size without killing the microorganisms. The result was the development of the Biodetection Grinder, a device that employs a shearing action with a minimum energy input to generate the particles. Using this device the desired particle sizes were obtained in materials ranging from soft plastics to hard rocks.

This report describes the development of the prototype Biodetection Grinder, its operation and results of laboratory evaluation of selected aerospace components. A second generation version of the grinder was constructed and is presently in use at the Communicable Disease Center in Phoenix, Arizona. A production version, which modified the second generation type to provide faster particle reduction rates, was developed and transferred to the Communicable Disease Center for further evaluation.

DESCRIPTION OF OPERATIONS

These operating characteristics apply to the prototype, second generation and production grinder illustrated in Figures 1, 2 & 3.

Cutting or grinding of the specimen material is done under a slight pressure in a sealed transparent chamber. The specimen is held by a chuck type clamp mounted on a slide which moves horizontally back and forth. Coupling of the drive shaft to the slide is such that rotary motion of the drive shaft results in reciprocating motion to the slide and chuck. A 100 RPM 27 volt D.C. motor provides the driving power for the reciprocating chuck as well as the grinding wheel and the linear feed sub-assembly.

The grinding wheel drive shaft, motor and coupling arrangement provide a variable controlled linear feed of the grinder into the specimen material. The grinder is attached to the drive motor with an adaptor which permits quick disconnects for replacement purposes so that different grit size grinding wheels may be used.

Linear movement of the grinder and its drive motor sub-assembly is accomplished through a gear reduction mechanism and driven by a variable speed motor. The gear reduction mechanism controls the forward movement of the cutter to 0.0001 inches per revolution, and the total linear movement is from zero to one inch.

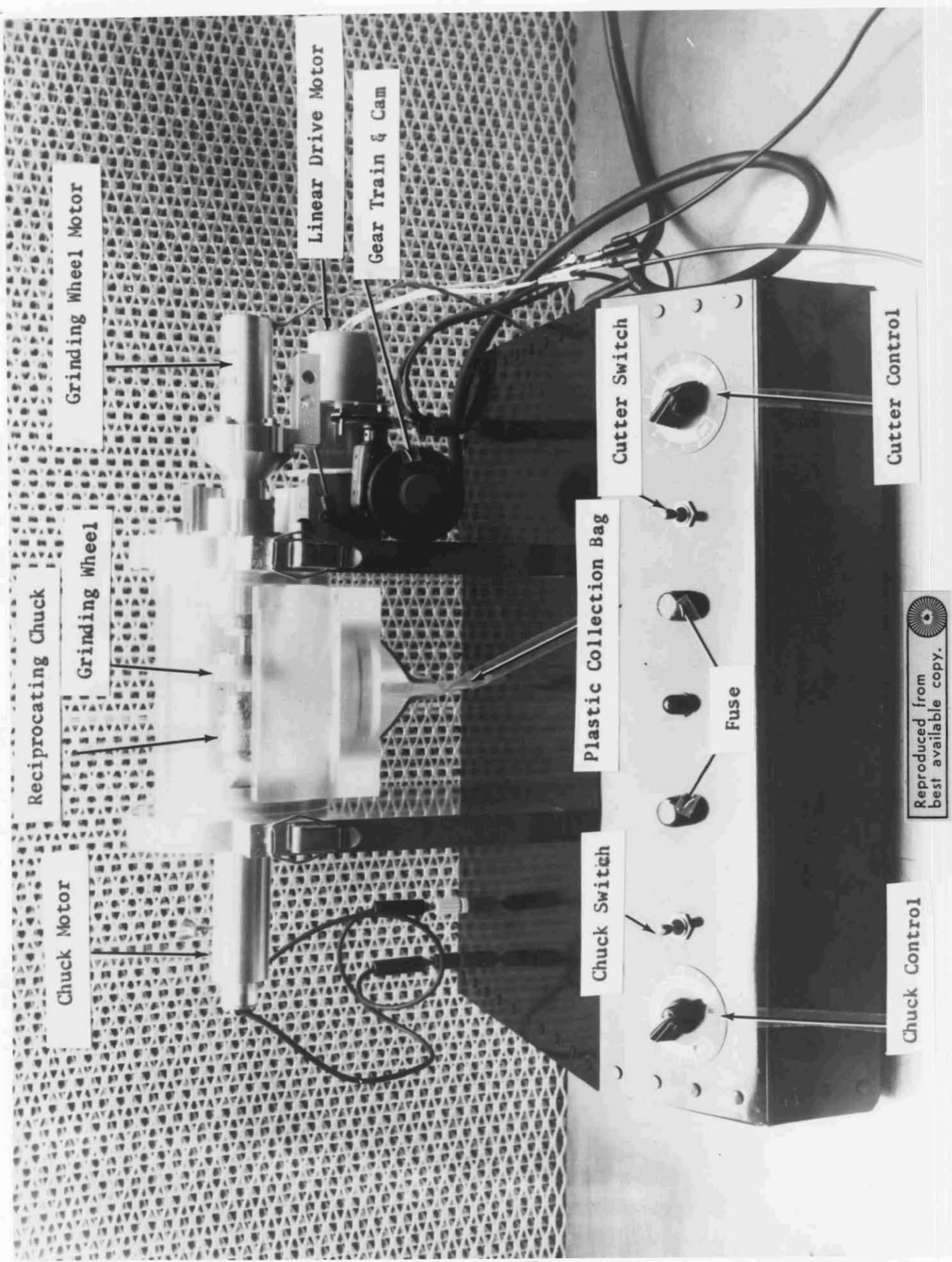
Fines from the sample may be collected in a polyethylene bag for aseptic storage as shown in Figure 1. The polyethylene bag may be replaced by a petri dish if immediate bio-assay of the fines is desirable.

Improvements in the second generation grinder shown in Figure 2 are as follows:

- (1) A limit switch arrangement is incorporated to provide automatic cut-off of linear motion at the desired increment settings.
- (2) A linear travel indicator shows the amount of material removed from specimens thereby eliminating the need for weighing fines.
- (3) An automatic sealing mechanism is incorporated to aseptically seal the polyethylene bag.
- (4) A port is provided in the top of the cutting chamber directly above the specimen to introduce sterile gas, air or cooling liquid directly on the specimen. An air stream from the port can be used to direct the flow of specimen fines toward the collector.

Specific changes incorporated in the production version shown in Figure 3 were:

- (1) The aseptic chamber was not needed and was replaced by a Plexiglas hood because the grinder was used in a laminar flow clean room.
- (2) The fines from the grinding wheel are collected directly in a sterile beaker eliminating the need for the particle collector and bag sealer. By removing the bag sealer, it was possible to



Note: Linear Motor Control in Rear

Figure 1. BIODETECTION GRINDER (PROTOTYPE)

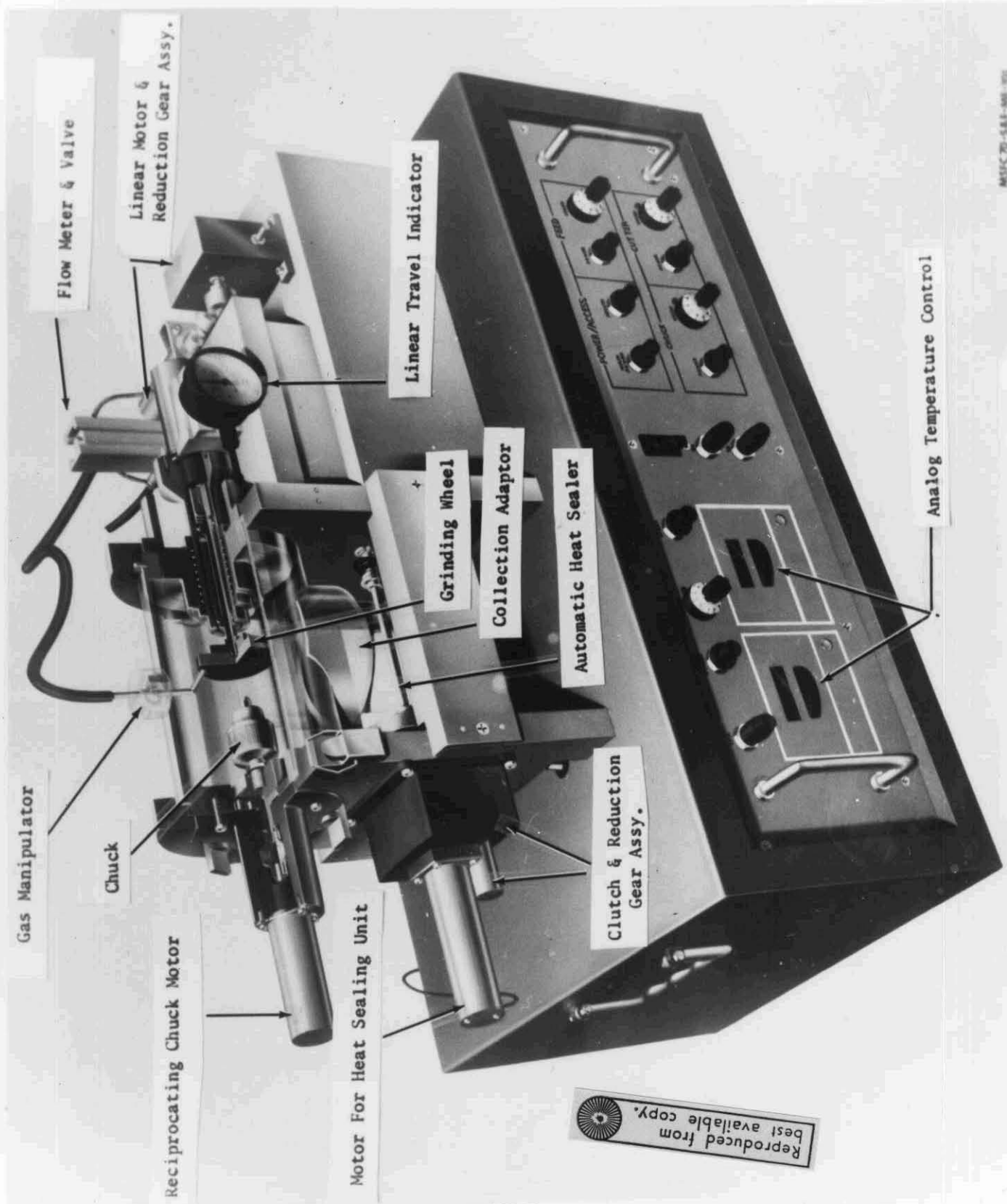


Figure 2. BIODETECTION GRINDER (SECOND GENERATION)

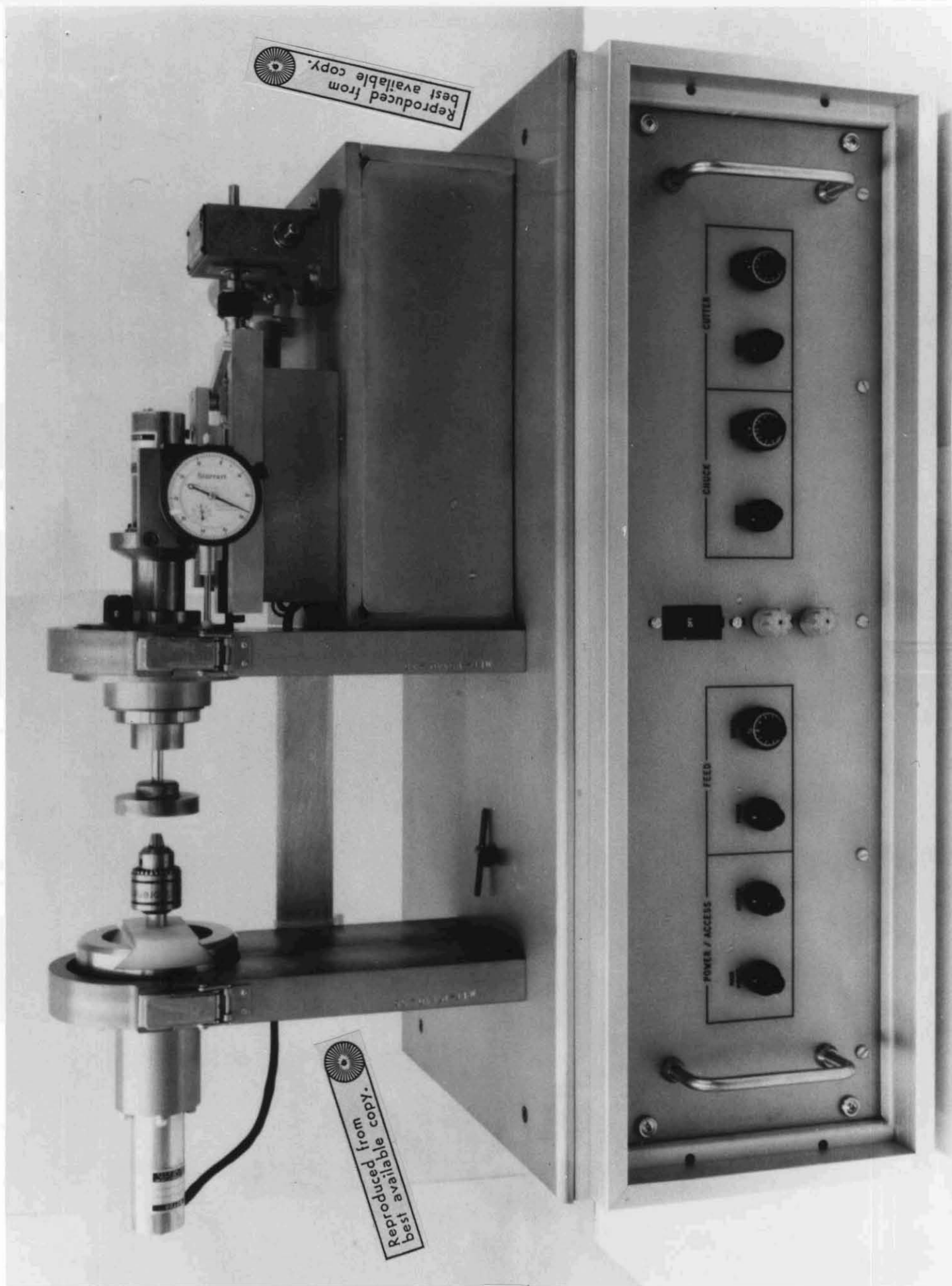


Figure 3. Biotetection Grinder (Production Version).

place an adjustable platform under the grinding wheel to support the beaker.

- (3) To reduce the time presently required to sterilize the cutting wheel and single arbor, several slip-in arbors and snap-on cutting wheels are used, permitting sterilization of several units at one time while several other units are in operation.
- (4) A micrometer-set automatic shut-off device was included to permit presetting the grinder to take a certain size sample at the completion of which the machine is automatically stopped.

MECHANICAL EVALUATION OF PROTOTYPE BIODETECTION GRINDER

A predictable size range of particles from a wide variety of materials is required for accurate analysis of embedded microorganisms. Tests were conducted to determine how well the Biodetection Grinder met this requirement. Nine specimens of varying hardness were ground and sized. The specimens were identified as follows:

<u>Specimen No.</u>	<u>Material</u>
1	Resistor, Brass Core
2	Resistor, Copper Core
3	Resistor, Micarta
4	Resistor, Steel Core
5	Aluminum Alloy 6061-T6

<u>Specimen No.</u>	<u>Material</u>
6	Brass, Hard Navy
7	Lucite
8	Rigidax
9	Resistors, Graphite Core

Attempts were made to grind two additional materials without satisfactory results. Grinding of Epoxy C-7 resulted in loading the cutting wheel with the specimen material thereby reducing the ability of the grinder to control particle size. Careful selection of grinding parameters reduces the wheel loading problem, but further research is needed to provide satisfactory grindings for materials of this nature.

Silicone rubber (RTV 11) is an extremely resilient material. During grinding, this material tends to break up into relatively large clumps. Restraining this specimen in a rigid tube results in a controlled grinding process. It should be noted that the use of a tube restraint introduces a contaminant into the specimen material. Therefore, care should be exercised in selecting a restraint material that is non-bacteriostatic.

Table 1 shows the distribution of particles sizes for the nine materials ground.

TABLE I
PARTICLE SIZE DISTRIBUTION IN PERCENTAGE

Specimen Number	Particle size range in microns					Cutter Speed RPM	Chuck Speed Str. Per. Min.
	>100	50-100	25-50	5-25	3-5		
1	0.13	0.50	1.92	38.12	59.33	12	10
2	0.5	0.25	1.37	41.59	56.29	15	10
3	0.40	0.84	3.47	38.51	56.76	25	20
4	0	0.21	0.99	20.38	77.69	15	10
5	4.25	0.53	3.72	21.27	70.21	12	10
6	7.17	2.05	2.73	23.84	64.16	25	10
7	0.57	0.23	0.86	39.23	59.11	30	20
8	5.26	6.58	3.29	47.37	37.50	30	15
9	0	0.45	1.37	37.50	60.68	12	10

All tests were made with a linear feed pressure of 2.5 pounds and a cutting wheel of 100 grit.

BIOLOGICAL EVALUATION OF SAMPLES PRODUCED FROM THE BIODETECTION GRINDER

Section I

In order to establish baseline data for the Biodetection Grinder, biological model systems were made from Rigidax, a soft water soluble plastic, Lucite a hard acetone soluble plastic and Castoglas, a hard clear insoluble plastic.

Pellets made of Rigidax and Lucite were impregnated with spores of Bacillus subtilis var. niger, assayed for uniform spore distribution, and ground

in the Biodetection Grinder. The grindings were cultured and percent recovery calculated. Grindings were also measured and size percentage ranges were established for the number 100 grit grinding wheel.

The castoglas pellets embedded with Bacillus subtilis and Bacillus stearothermophilus impregnated spore strips were ground on the Biodetection Grinder. The grindings were cultured and percent recovery calculated. Several spore strips were cultured individually to determine the actual number of spores impregnated on each strip. This determination was used as a baseline to calculate the percent of microorganisms recovered from the grindings.

A. Pellet Formation

1. Rigidax is a semi-hard, gray water soluble plastic which melts at 70°C and sets at about 40°C. Pellets contaminated with spores of Bacillus subtilis were prepared in a class 100 clean bench by weighing ten (10) grams of Rigidax in an aluminum pan. The lyophilized spore suspension was suspended in 1 ml 95% ethanol and ultrasonicated to break up clumps. This was verified by spore stains.

Rigidax was melted and the spore suspension was added. A sterile wooden stick was used to stir the mixture which was allowed to bubble gently until evaporation of the ethanol appeared complete. Overheating was avoided so that maximum spore survival was attained. Liquid Rigidax was immediately poured into No. 00 gelatin capsules and placed in a refrigerator at 4°C to harden thoroughly.

After two hours the pellets were removed from the refrigerator, scored with a sterile, heated scalpel and the gelatin covering discarded. The

Rigidax pellet was handled with a gloved hand and transferred to the freezer for storage at -15°C in sterile plastic bags.

2. Lucite is a hard, clear acetone soluble plastic which is made from two components and set at 50°C over a period of six (6) hours.

Pellets approximately 1/4 inch in diameter were prepared in a class 100 clean bench as follows:

Removal of Inhibitor

Methyl Methacrylate Liquid (MML) contains a polymerization inhibitor which was removed in the following manner:

One (1) portion of MML was washed twice in the separatory funnel with two successive portions of 2% NaOH. The first washing gave a dirty pink color; the second wash was clear.

The MML was then washed with two successive portions of distilled water. The MML forms the top layer in each case.

The MML was then transferred to a glass stoppered bottle and 10 gm Na_2SO_4 was added per 100 ml of washed MML to remove any remaining water. This mixture was stored at 4°C until needed.

Contamination of MMP (Methyl Metacrylate Powder)

Then (10) grams of MMP was weighed in an aluminum pan. A lyophilized suspension containing 6×10^6 Bacillus subtilis spores was suspended in 95% ethanol and ultrasonicated 12 min. to break up clumps of spores. Absence of clumping was verified by spore stains. Operating in the clean bench, the spore suspension was added to the ten (10) grams of MMP, mixed with a sterile wooden stick, and allowed to dry for 16 hours in the clean bench.

Preparation of mixture of MMP and MML

When the contaminated MMP was thoroughly dry, eight (8) ml of the MML was added and stirred gently with a sterile wooden stick. This mixture was placed in a vacuum jar and 28 in.hg vacuum was pulled slowly, causing bubbles of gas to evolve from the plastic. When bubbles ceased to evolve, the mixture was removed and gently stirred to make a clear casting. The mixture was poured in No. 00 capsules and placed in a 50°C oven for six (6) hours.

The pellets were placed in sterile water to allow the capsules to dissolve. The pellets were removed and stored in sterile plastic bags at -15°C.

3. Clear, hard, insoluble plastic and castoglas pellets were contaminated with spore strips. One spore strip was rolled into a circle and placed in the bottom of each gelatin capsule. Castoglas, made according to directions on the can, was poured into each capsule and allowed to harden for 2 1/2 hours at room temperature. The gelatin capsules were then removed with a heated scalpel. Each pellet was put into a separate plastic bag and stored in the refrigerator until needed.

B. Collection and Treatment of Grindings and Pellets

1. Metrology - pellets - Each pellet was weighed on a balance with a 0.001 gm sensitivity and measured with a micrometer.

2. Storage - pellets - Each pellet was identified in separate bags by a number, weight, and length; and stored at -15°C until needed.

3. Metrology - grindings - Grindings were collected in pre-weighed plastic bags so that the weight of the grindings could be determined after collection without removal from the collection bags. The grindings were stored at -15°C until cultured.

4. Metrology - Pellet remaining after grinding - The Lucite and Rigidax pellets remaining in the chuck after grinding were weighed, measured, and stored in an identified plastic bag at -15°C until ready for culture. It was not necessary to weigh the remaining castoglas pellets since the material was insoluble and could not be cultured.

C. Culture Techniques

1. Rigidax - to determine uniform spore distribution throughout the Rigidax pellets, two pellets were cut into two pieces. Each piece was weighed, then dissolved in sterile distilled water to give a 1:100 dilution. Serial 1:100 dilutions were made and plated in duplicate by standard pour plate methods.

Seven Rigidax pellets were ground by the Biodetection Grinder. The grindings and the pellets remaining after grinding were dissolved in sterile distilled water according to weight, to give 1:100 dilution. Serial 1:10 dilutions were made and plated in duplicate by standard pour plate methods.

Colony counts were determined on all pour plates after 24 hours incubation at 35°C.

2. Lucite - To determine uniform spore distribution throughout the Lucite pellets, four pellets were cut into two pieces. Each piece was weighed then dissolved by weight in sterile acetone to give a 1:100 dilution. Serial 1:10 dilutions were made and plated in duplicate by standard pour plate methods.

Colony counts were determined on all pour plates after 48 hours incubation at 35°C.

3. Castoglas - To determine the number of spores impregnated on the spore strips embedded in the castoglas pellets, three spore strips were placed individually into ten ml of trypticase soy broth, ultrasonicated for twelve minutes and incubated for three hours at 35°C. After incubation, serial dilutions of 10^3 , 10^4 and 10^5 were made and 1 ml of each dilution was plated in duplicate with trypticase soy agar. The number of colonies was counted after 24 hour incubation at 35°C. These colony counts were used as a base line for determining the percent recovery from the grindings.

The end of the pellet containing the rolled spore strip was completely ground. One tenth gram of the grinding was placed into ten ml of trypticase soy broth and cultured (in the same manner as the spore strips in the preceding paragraph). The colony count data obtained from the spore strips and the grindings were used to compute the percent recovery.

D. Particle Sizing of Rigidax and Lucite

1. Slide Preparation - After grindings had been removed for culturing a small amount of grindings were suspended in five (5) ml of freon and ultrasonicated for one (1) minute to disperse clumps. A slide was prepared from grindings representative of each pellet by placing one (1) drop of the ultrasonicated material on alcohol flamed slide and allowing the freon to evaporate. This was covered with a flamed coverslip and microscopically examined at 500X.

2. Microscopic Sizing - Particles were sized and percentages calculated by measurement with a calibrated ocular micrometer at 500X. Particles were sized from 2-4 microns, 4-8 microns, and greater than 8 microns. The number of particles counted on the thinnest section of each slide was one hundred (100).

RESULTS

A. Rigidax

It was found that spores had been uniformly distributed throughout the pellets assayed for this purpose. (See Table II)

Recovery of spores from grindings compared with recovery from the remaining pellet is shown in Table III.

Size range of the grindings is shown in Figure 4.

TABLE II. ASSAY OF RIGIDAX PELLETS TO SHOW UNIFORM DISTRIBUTION

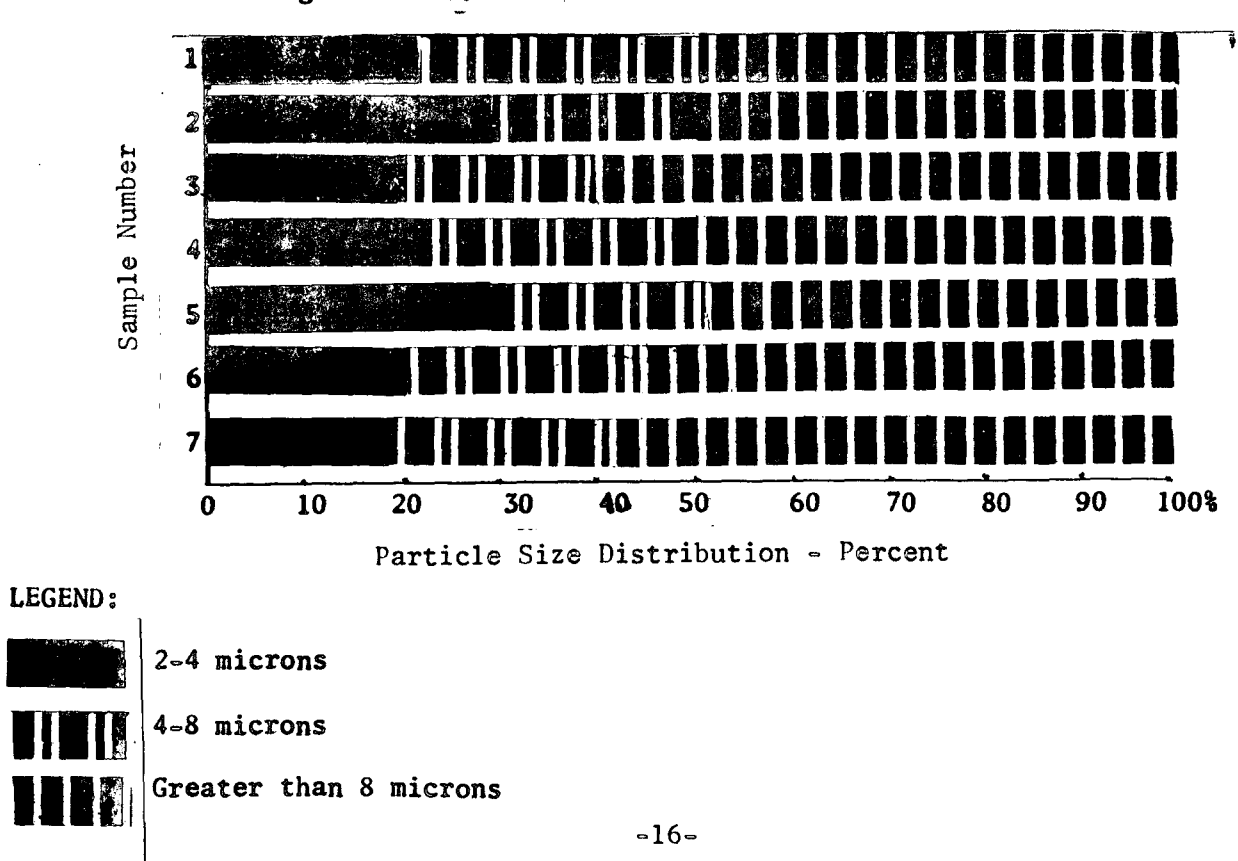
Sample	Replicate	Standard Pour Plate Count	Average Pour Plate Count
1 a*	1	5.52×10^6	5.56×10^6
	2	5.60×10^6	
1 b*	1	5.40×10^6	5.37×10^6
	2	5.34×10^6	
2 a*	1	5.78×10^6	5.55×10^6
	2	5.33×10^6	
2 b*	1	5.57×10^6	5.51×10^6
		5.46×10^6	

* a and b refer to sections of the same pellet.

TABLE III. PERCENT RECOVERY OF ORGANISMS FROM RIGIDAX CAPSULES AFTER GRINDING

Sample	Pellet After Grinding Standard Pour Plate Count	Grindings - Standard Pour Plate Count	Percent Recovery of Organisms
1	7.00×10^6 7.40×10^6	6.70×10^6 6.69×10^6	93%
2	6.90×10^6 6.69×10^6	6.54×10^6 6.25×10^6	94%
3	8.26×10^6 7.83×10^6	8.03×10^6 7.98×10^6	99%
4	6.18×10^6 5.60×10^6	5.38×10^6 5.16×10^6	89%
5	7.31×10^6 6.97×10^6	7.26×10^6 7.10×10^6	100%
6	5.56×10^6 5.49×10^6	5.32×10^6 5.30×10^6	96%
7	6.13×10^6 6.27×10^6	5.80×10^6 5.46×10^6	92%

Figure 4. Percent Distribution of Grindings in Microns Size Range of Rigidax Grindings With No. 100 Wheel



B. Lucite

Cultures of pellets cut in pieces indicate that spore distribution in Lucite varied throughout the pellet as shown in Table IV.

Spore recovery from grindings compared with recovery from the remaining pellet is shown in Table V.

Size range of the grindings is shown in Figure 5.

TABLE IV: Assay of Lucite Pellets to Show Uniform Distribution.

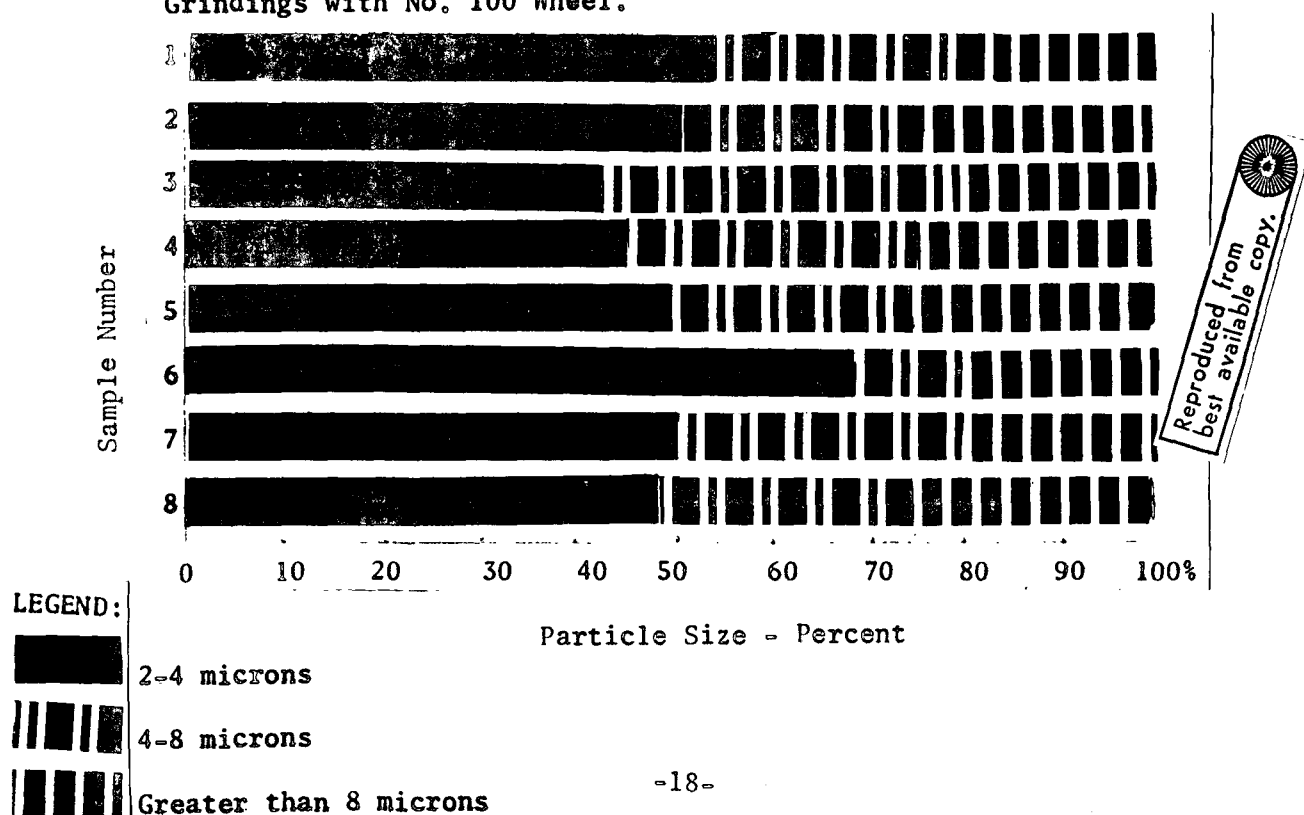
Sample	Replicate	Standard Pour Plate Count	Average Pour Plate Count
1 a*	1	1.17×10^6	1.20×10^6
	2	1.23×10^6	
1 b*	1	1.00×10^6	1.03×10^6
	2	1.05×10^6	
2 a*	1	1.70×10^5	1.40×10^5
	2	1.10×10^5	
2 b*	1	3.3×10^5	3.7×10^5
	2	4.1×10^5	
3 a*	1	8.3×10^5	9.0×10^5
	2	9.7×10^5	
3 b*	1	10.0×10^5	9.9×10^5
	2	9.8×10^5	
4 a*	1	7.2×10^5	7.1×10^5
	2	7.0×10^5	
4 b*	1	3.6×10^5	3.4×10^5
	2	3.1×10^5	

* a and b refer to sections of the same pellet.

TABLE V. Percent Recovery of Organisms From Lucite Capsule after Grinding

SAMPLE	Pellet After Grinding - Standard Pour Plate Count	Grindings - Standard Pour Plate Count	Percent Recovery of Organisms
1	8.0×10^5 7.4×10^5	1.9×10^5 2.1×10^5	26%
2	6.4×10^5 7.2×10^5	2.1×10^5 2.6×10^5	33%
3	6.8×10^5 7.5×10^5	6.0×10^4 6.0×10^4	8%
4	5.8×10^5 6.0×10^5	2.6×10^5 3.0×10^5	47%
5	2.0×10^5 1.9×10^5	1.0×10^5 1.2×10^5	50%
6	4.7×10^5 4.2×10^5	3.0×10^4 3.0×10^4	7%
7	5.5×10^5 4.8×10^5	8.0×10^4 9.0×10^4	17%
8	4.0×10^5 3.9×10^5	1.9×10^5 2.3×10^5	53%

Figure 5: Percent Distribution of Grindings in Microns Size Range of Lucite Grindings with No. 100 Wheel.



C. Castoglas

Since the entire spore strip in the end of each pellet was ground, the pellet was considered to have a uniform distribution of organisms.

Table VI is included to indicate the average pour plate count for three spore strips that were not embedded in castoglas.

Percent recovery of the ten samples tested varied with each pellet as shown in Table VII.

TABLE VI. Assay of Spore Strips to be used as a Baseline for Determining the Percent Recovery from Castoglas Pellets

SAMPLE	REPLICATE	STANDARD POUR PLATE COUNT	AVERAGE POUR PLATE COUNT
1	1	2.3×10^5	2.4×10^5
	2	2.5×10^5	
2	1	2.15×10^5	2.08×10^5
	2	2.02×10^5	
3	1	2.43×10^5	2.35×10^5
	2	2.27×10^5	

TABLE VI: Pellets Percent Recovery of Organism from Castoglas after Grinding

SAMPLE	REPLICATE	STANDARD POUR PLATE COUNT X 10 ⁵	AVERAGE POUR PLATE COUNT X 10 ⁵	PERCENT RECOVERY
1	1	1.10	1.08	47.4
	2	1.06		
2	1	.91	.905	39.6
	2	.90		
3	1	1.36	1.29	56.6
	2	1.23		
4	1	.97	.935	41.0
	2	.90		
5	1	.68	.69	30.3
	2	.70		
6	1	.71	.725	31.8
	2	.74		
7	1	.43	.455	20.0
	2	.48		
8	1	1.45	1.43	62.7
	2	1.41		
9	1	.88	.855	37.5
	2	.83		
10	1	.94	.965	42.3
	2	.99		

DISCUSSION

A. Rigidax

Rigidax is an easily impregnated material which, when ground, produces a high percentage of greater than 8 micron size particles. This was expected because Rigidax has a tendency to flake even when ground slowly. Reproducibility

of particle size was good.

Spore distribution was uniform throughout the pellet. This was reflected by the consistency of recovery of high percentages of organisms from the grindings.

B. Lucite

Lucite was more difficult than Rigidax to uniformly impregnate with spores. This was seen by the results of assaying pellets cut in half. The erratic results obtained from cultures of the grindings was most likely due to non-uniform impregnation of the Lucite pellets. Lucite produced a high percentage of particles in the 2-4 micron range.

C. Castoglas

Spores were uniformly distributed in the Castoglas pellets by the incorporation of commercial spore strips in the ends of the pellets.

The variability of the percent recovery of the spores from the castoglas was possibly due to the insolubility of castoglas. Some spores remained trapped in the plastic where they could not come in contact with the nutrient media. Thus, the spores could not germinate.

CONCLUSION

Test results using the Biodetection Grinder and three biological model systems show that the Biodetection Grinder can produce grindings in a predictable size range. Further, these results indicate that reproducible recovery rates are dependent on the type of material used for making the biological test models.

Additional tests should be performed to produce uniformly contaminated biological models using different materials to demonstrate the efficiency of the Biodetection Grinder. While work is being carried out to produce these models, actual spacecraft components can be ground and assayed for viable organism content.

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APPENDIX I

OPERATION PROCEDURE FOR BIO- DETECTION GRINDER

SCOPE

This set of procedures is designed to instruct assay laboratories in the aseptic operation of the Biodetection Grinder using materials contaminated with spores of Bacillus subtilis. These instructions are based on operating procedures currently in use by this laboratory for the Prototype Grinder.

EQUIPMENT

Tools needed for the operation, adjustment and cleaning of the grinder are as follows:

Jacobs chuck key 1/4 inch

Six inch screw driver with a 1/4 inch blade

Wire brush

1/2 inch round camel hair brush

Equipment utilized for maintenance of aseptic techniques and contamination control are:

Class 100 clean bench

Ethylene oxide sterilizer

Sterile forceps

Sterile 4 mil polyethylene bags 3 1/4 inches wide x 3 inches long

Sterile gloves

Sterile screwdriver

Sterile 1/4 inch jacobs chuck key

Formaldehyde 5% in 70% isopropyl alcohol

Sterile towel 18 x 18 inches

Rodac plates filled with trypticase soy agar

Isopropyl alcohol 70%

Unispore spore strips

Biodetection Grinder

GRINDER ASSEMBLY

Fit chuck-motor assembly in one end of grinding chamber.

Attach appropriate grit grinding wheel to shaft with three (3) screws (provided).

Insert shaft into grinder-motor housing and push with a twisting motion until a click is heard.

Fit grinder-motor housing assembly into the end of the grinding chamber opposite the chuck.

Place grinding collection receptacle in the hole in the bottom of the grinding chamber.

Insert spore strip impregnated with Bacillus Subtilis spores in the opening for the collection bag.

Place the entire assembly in a 4 mil plastic bag 24 inches by 8 inches. Insert a cotton wick in the end, secure with a rubber band and a piece of ethylene oxide sensitive tape.

STERILIZATION

The assembled grinding chamber is sterilized using 12% ethylene oxide and 88% freon in an air displacement cycle at 130°F for 16 hours.

After completion of the sterilization cycle the unit (in its sterilization bag) is placed in a class 100 clean bench with the blower operating and allowed to degas for 24 hours.

Polyethylene bags 3 1/2 x 3 inches, 4 mil thick are packaged singly in paper pouches and sterilized with the grinding chamber and degased.

A spore strip is inserted in the paper pouch prior to sealing.

All other sterile tools are packaged in one paper pouch and sterilized as specified above.

ASEPTIC INSERTION OF BIO-MODEL PELLET

Wipe the inside of the class 100 clean bench with 70% isopropyl alcohol to decontaminate.

Four Rodac plates are placed in the clean bench.

The sterile towel is placed on the work surface of the clean bench.

The bag containing the grinding chamber is opened in the clean bench and the chamber placed on the sterile towel.

The bags containing sterilized tools and collection bag are opened and the contents placed on the sterile field.

The specimen to be ground is aseptically removed from its storage bag and placed on the sterile field.

Sterile gloves are donned.

All spore strips are removed and cultured.

Chuck-motor assembly is aseptically removed using the sterile screw driver.

Place specimen in chuck and tighten chuck with sterile jacob's chuck key.

Replace chuck-motor assembly in end of grinding chamber.

Place specimen bag on specimen collector.

Remove sterile gloves and sterile assembly material.

The grinder chamber is now handled as a closed sterile item.

THE GRINDING OPERATION

Check to see that all power and drive switches are off.

Plug power cords into 115 volt receptacles and grinder power source.

Turn on all main power switches.

Adjust speed and feed controls to the desired rates.

Grind specimen.

Shut off all switches, disconnect power cords.

Remove grinder chamber top mounts.

Loosen grinder connector screw.

Remove grinder chamber from power source.

Place grinder chamber on towel.

Remove collection bag using aseptic technique, fold top edge in and seal with scotch tape.

Remove chuck-motor housing and loosen chuck.

Using sterile forceps, transfer remainder of specimen back to its storage container.

PREPARATIONS FOR REASSEMBLY OF GRINDING CHAMBER

Using screwdriver, remove grinder-motor housing.

Use the 1/2 inch camel hair brush and remove any particles of the specimen adhering to parts of the grinder or chamber. Be certain that all excess grindings cleaned from the mechanism are collected on the towel.

After cleaning the housing, use the wire brush to clean the grinding wheel. Reassemble grinder.

Remove towel from the work area and autoclave.

Wipe all surfaces in the clean bench with the formaldehyde/alcohol mixture.

QUALITY ASSURANCE

Spore strips impregnated with Bacillus Subtilis spores and packaged in glassine envelopes (Unispore, Castle Co.) are included in each separate package in articles to be sterilized by ethylene oxide.

Spore strips are removed from the sterilized packages and cultured according to manufacturer's instructions.

Rodac plates filled with trypticase soy agar are placed in the clean bench to aid in detection of external contamination.

If the sterile assembly-grinding procedure requires longer than one (1) hour, Rodac plates are replaced at the rate of four (4) per hour to prevent excessive drying of the culture medium.

APPENDIX II

A. Controls

1. Spore strips impregnated with 1×10^8 Bacillus subtilis were included in each sterilizer run of Biodetection Grinder parts.

B. Sterilization

1. Parts of the Biodetection Grinder were cleaned and placed in 4 mil polyethylene bags with a cotton wick in the end. The parts were sterilized with 12% ethylene oxide and 88% freon using a 16 hour air displacement cycle. Sterilization was accomplished after each grinding period.

2. All other equipment and materials were sterilized using the 16 hour air displacement cycle with 12% ethylene oxide and 88% freon sterilant combination. Before any equipment was used after sterilization, it was allowed to outgas for 24 hours.